

HORSES AND OTHER EQUIDS

Exophiala phaeomuriformis associated with abortion and placentitis in a mareSimon de Neck,¹ Ben Jurgens,¹ Liana Peters,² Nynke Ankringa¹

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SUMMARY

This article describes a case of abortion and placentitis in a mare. Macroscopic and microscopic lesions in the aborted fetus were consistent with a pyogranulomatous inflammation with intralesional fungal structures. These were observed in the lungs, skin, stomach, umbilical cord, peritoneum, fetal membranes and brain. Molecular techniques detected the fungus *Exophiala phaeomuriformis* from those lesions. To the authors' knowledge, this is the first reported case of abortion in horse due to *E. phaeomuriformis*.

BACKGROUND

This case report describes a case of placentitis and abortion in a mare, associated with *Exophiala phaeomuriformis*, and outlines the diagnostic method leading to this diagnosis. To the authors' knowledge, there have not been previously described cases of equine abortion and placentitis due to *Exophiala* species.

CASE PRESENTATION**History**

In April 2016, a 17-year-old Royal Dutch Sport Horse (KWPN) mare (mare 1) (*Equus ferus caballus*) was bred to a 14-year-old KWPN stallion by means of artificial insemination. This mare was diagnosed as pregnant on May 18, 2016, after a second artificial insemination. Ultrasonographic follow-up examinations of the gestation were performed on the 21st, 45th and 68th days of gestation and no abnormalities were observed. In November 2016, another mare (mare 2) from the same stable aborted at 31 weeks of gestation. At that stage, blood was collected from both mares (mares 1 and 2) and sent for equine herpesvirus 1 (EHV-1) investigation, with the addition of several other organs from the aborted fetus (liver, lungs, kidney and placenta). The PCR results of these organs were negative for EHV-1. There were no previous known cases of abortion in this stable. Mare 1 was not moved between stables for the previous five years, including the current gestation, and had previously foaled uneventfully as a 3, 4 and 5-year-old. This first mare was presented on December 21, 2016, to the veterinarian for colic.

Clinical examination

On clinical examination, mare 1 was pyrexia (38.9°C), was sweating and the fetal membranes were visible at the vulva. The fetus was in correct presentation but showed no signs of life. The fetus

and membranes were removed. At the time of abortion, the mare had no udder development.

The aborted fetus was sent for necropsy to the Veterinary Pathology Diagnostic Centre, Faculty of Veterinary Medicine, Utrecht University, The Netherlands.

INVESTIGATIONS**Macroscopic findings**

The female fetus weighed 14 kg and measured 60 cm from crown to rump, with normal fat deposits and musculature for the age. Disseminated in the skin (Fig 1a), umbilical cord and fetal membranes (Fig 1b) (mainly surrounding the large blood vessels close to the umbilical cord) were moderate to large numbers of well-demarcated, elevated, firm, grey/green nodules measuring 3–4 mm in diameter.

In the chorion and allantois, these nodules were surrounded by a well-demarcated soft, green ring of approximately 1 cm in diameter with a dull aspect (Fig 1b). The blood vessels of the amnioallantois were severely swollen and tortuous.

The lungs were diffusely firm and pale (Fig 1c) with disseminated, both on surface and cut surface, similar nodules as those described for the fetal membranes and skin.

Focally extensive in the omentum between the stomach and the spleen, and focally in the gastric wall, large numbers of identical nodules were present. At this level, the gastric mucosa of the glandular part was focally extensive, indented and dark green, showing a sharp demarcation with the non-glandular part.

The other organs showed no significant macroscopic changes.

Cytology

The cytology smear of the lungs (Hemacolor stain) (Fig 1d) was well stained, moderately cellular, with few erythrocytes, and abundant pale bluish, finely granular background (protein-rich fluid). The majority of the cells consisted of macrophages, together with low numbers of eosinophils, lymphocytes, neutrophils and fibroblasts. Disseminated throughout the smear, moderate numbers of extracellular fungal structures were present and characterised by septate, non-branching hyphae. The fungi ranged from non-stained to bluish.

Histopathology

On haematoxylin and eosin stain, similar lesions were observed in the lungs, skin, stomach, cerebral cortex and amnioallantois (Fig 2). In these



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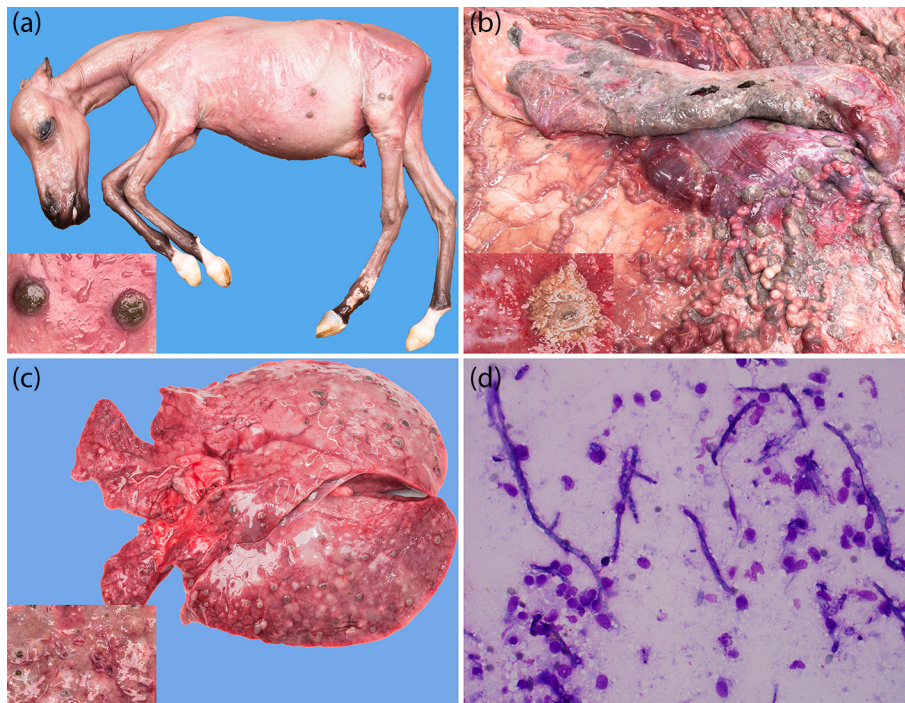


FIG 1: (a) Aborted fetus. In the skin, multifocally, grey to green nodules. Inset: cutaneous nodules. (b) Amnioallantois and umbilical cord. Tortuous blood vessels of the amnioallantois with, disseminated in both the amnioallantois and the umbilical cord, grey to green nodules. Inset: chorioallantois. Nodule surrounded by a green, dull, irregular ring. (c) Lungs. Firm and pale lungs with, disseminated, grey to green nodules. Inset: cut surface of the lung. (d) Cytology of the lungs. Macrophages and septate hyphae. Hemacolor. x 400.

organs, the tissue architecture was disrupted by single (cortical grey matter) to several variably sized granulomas characterised by abundant, faintly stained, 3 µm width septate hyphae, and oval to ellipsoid structures of 6–7 µm in diameter (yeast-like structures) (fungi), admixed with moderate numbers of neutrophils (with exception of the brain), macrophages and moderate amount of granular eosinophilic materials and karyorrhectic debris (necrosis). This central necrotic area was surrounded by concentric layers of fibrillary eosinophilic material (collagen), large numbers of elongated/spindle-shaped cells (fibroblasts) (fibrosis) and large numbers of multinucleated giant cells with up to 10 eccentric nuclei (Langhans type), with abundant granular eosinophilic cytoplasm, and occasionally containing fungi.

In the lungs, the alveoli surrounding the granulomas contained moderate numbers of neutrophils (viable and degenerated), macrophages, eosinophils, multinucleated giant cells, fungal structures and moderate amounts of necrotic cellular debris. The interstitium was markedly thickened by moderately dense connective tissue (fibrosis) multifocally expanded by clear spaces between the collagen fibrils (moderate oedema), occasional fungal hyphae, small haemorrhages and mild perivascular oedema.

Between the granulomas, the amnioallantois stroma was markedly oedematous and expanded by moderate amount of amorphous eosinophilic beaded materials (fibrin), large amount of eosinophilic amorphous to granular materials and moderate amount of karyorrhectic nuclei (necrosis), and basophilic granular materials (mineralisation), with disseminated moderate numbers of neutrophils and macrophages, and large numbers of fungi. Some small blood capillaries contained amorphous eosinophilic materials in their lumen (microthrombi). The skin and stomach displayed similar lesions, with necrotic areas being superficial and associated with ulceration.

The umbilical cord and the chorioallantois lacked discrete granulomas. Instead, the fungi were associated with large areas of necrosis which were demarcated from the surrounding viable tissue by moderate numbers of neutrophils, macrophages and multinucleated giant cells (Langhans type). In the chorioallantois, the necrosis involved the overlying chorioallantoic villi. The viable tissue displayed marked hyperaemia, moderate oedema and multifocally small haemorrhages. Some small blood capillaries with microthrombi were seen. In the umbilical cord, some blood capillaries showed a hypereosinophilic wall (degeneration/necrosis) with intraluminal fungi. The Wharton's jelly had small numbers of neutrophils and lymphocytes around the blood vessels.

Summary of the pathological findings

The aborted fetus had a pyogranulomatous and eosinophilic pneumonia, a granulomatous encephalitis, a pyogranulomatous gastritis, dermatitis, peritonitis, placentitis and omphalitis, with necrosis in the umbilical cord, chorioallantois, amnioallantois, skin and stomach. Fungi were observed on histopathology in the lungs, cerebral cortex, stomach, skin, umbilical cord, chorioallantois and amnioallantois. On histopathology, the fungus forms light yellow yeast-like structures and septate hyphae.

Additional stains

The described fungal elements stained with both Grocott's methenamine silver stain (Fig 2a) (inset) and periodic acid-Schiff stain (Fig 2c) (inset).

Fungus identification

A pooled sample of lung, chorioallantois, amnioallantois and umbilical cord was sent for fungus identification (Westerdijk

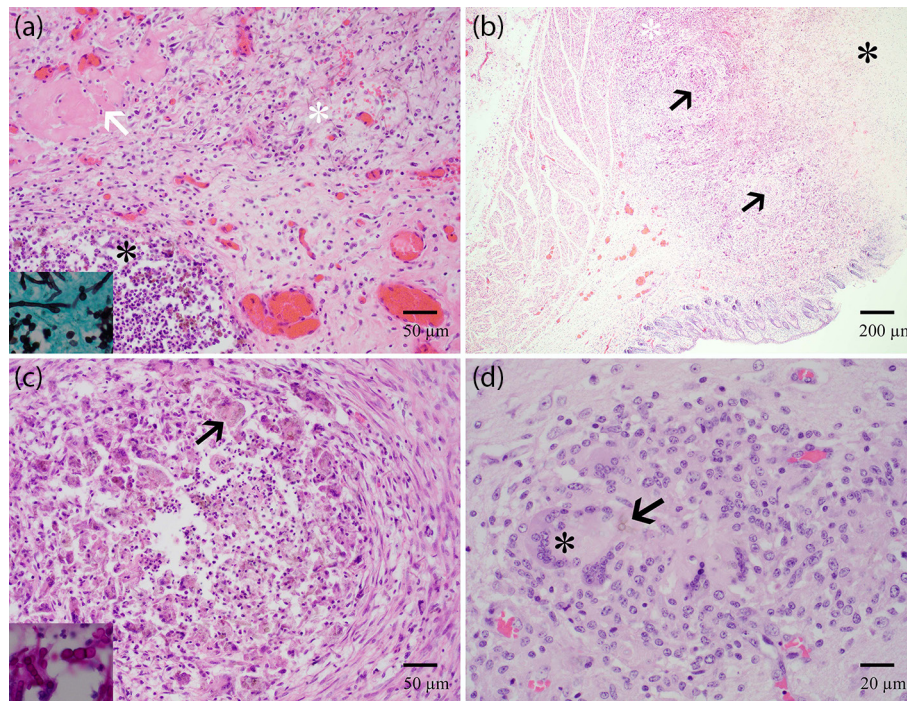


FIG 2: (a) Amnioallantois. Pyogranulomatous inflammation characterised by a granuloma containing macrophages, neutrophils, some karyorrhetic nuclei and fungi (black asterisk), and oedematous stroma with disseminated moderate numbers of macrophages and fungi (white asterisk). Focally, fibrin deposition (white arrow). The blood vessels are hyperaemic. Haematoxylin and eosin (HE). x 200. Inset: Grocott's methenamine silver (GMS) stain. Septate hyphae and yeast-like structures. x 400. (b) Skin. Pyogranulomatous dermatitis characterised by a severely expanded dermis containing granulomas (black arrows), with between large numbers of multinucleated giant cells (Langhans type), macrophages, neutrophils, fungi and a moderate amount of karyorrhetic nuclei (white asterisk). The superficial dermis consists of a large area of necrosis with abundant fungi and overlying ulceration (black asterisk). HE. x 40. (c) Lung. Pyogranulomatous and eosinophilic pneumonia. Granuloma characterised by moderate numbers of neutrophils and fungi surrounded by large numbers of multinucleated giant cells containing intracytoplasmic fungi (black arrow). HE. x 200. Inset: periodic acid-Schiff (PAS) stain. Septate hyphae and yeast-like structures. x 400. (d) Brain. Granulomatous encephalitis. Focal granuloma characterised by moderate numbers of macrophages, some multinucleated giant cells (black asterisk) and few fungi (yeast-like structure) (black arrow). HE. x 400.

Fungal Biodiversity Institute, Utrecht, The Netherlands). Culture was first attempted, but the morphological and colony characteristics were not specific enough for identification. Therefore, DNA material was extracted from the colonies and amplified via PCR assay. The isolated DNA sequence was 100 per cent identical to the fungus *E. phaeomuriformis*.

DISCUSSION

Fungal infections have been reported as a cause of abortion and stillbirth in horses, accounting for up to 15 per cent of the causes of placentitis in one study.¹ The reported fungi in that study were *Aspergillus* species and *Absidia* species. In another study, mycotic placentitis was mostly observed during late gestation with reported fungi being, in addition to *Aspergillus* species, *Candida* species and *Histoplasma* species.² Other studies also reported *Histoplasma capsulatum*,³ *Candida guilliermondii*⁴ and *Cryptococcus* species^{5,6} as a cause of placentitis and abortion in mares.

In the present study, a case of equine abortion due to fetal infection by *E. phaeomuriformis* is described. To the authors' knowledge, this fungus has not been previously described as a possible cause of abortion and placentitis in the horse.

The microscopic lesions described in this case are similar with previous reports of fungal placentitis in horses.²⁻⁶ The histological morphology of the fungus is not characteristic of *H. capsulatum* or *Cryptococcus* species. Therefore, by the mean of histopathology only, and based on the reported causes of fungal

placentitis in horses, suspected fungal aetiologies were *Aspergillus* species, *Absidia* species and *Candida* species. As reported before in skin lesions in cats,⁷ the identification of the fungus based on histopathology alone was not possible. Culture and PCR were required for the fungus identification, which in this study was *E. phaeomuriformis*. The lesions in this case are consistent with the ones reported in animals infected with *Exophiala* species.^{8,9}

Exophiala species is a ubiquitous melanised fungus within the order *Chaetothyriales*.^{10,11} On histology, this fungus has been described as pigmented irregular hyphae and yeast-like structures, both with septations.¹² Infections with this fungus have been reported in different species of fishes, crustaceans and reptiles,^{11,13-20} and it is known as a pathogen in mammals, causing cutaneous lesions in cats and dogs,^{7-9,21,22} with one case of systemic infection in a cat²³ and diseases in humans.²⁴ In cattle, *E. jeanselmei* has been isolated from one case of mycotic abortion.²⁵

The majority of equine placentitis, of bacterial or fungal origin, is the result of ascending infections.^{1,2} It occurs mostly in late gestation¹ and, in most cases, the infection is suspected to be caused by opportunistic microorganisms from the mare lower genital tract or from the environment.^{1,2} Due to the ubiquitous nature of *Exophiala* species, an ascending infection is considered the most likely route of entry in the case described in this paper.

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